

Amendments to the Specification:

Please replace the paragraph beginning at page 1, line 13 to page 2, line 10 with the following amended paragraph:

This application is related to U.S. application Serial No. 09/910,120, filed July 18, 2001, to Dana Ault-Riche and Paul D. Kassner, entitled "COLLECTIONS OF BINDING PROTEINS AND TAGS AND USES THEREOF FOR NESTED SORTING AND HIGH THROUGHPUT SCREENING", published as U.S. application Serial No. 20020137053, and to U.S. provisional application Serial No. 60/219,183, filed July 19, 2000, to Dana Ault-Riche entitled "COLLECTIONS OF ANTIBODIES FOR NESTED SORTING AND HIGH THROUGHPUT SCREENING". This application is related to International PCT application No. WO 02/06834. This application also is related to U.S. provisional application Serial No. 60/352,011, filed January 24, 2002, to Dana Ault-Riche and Paul D. Kassner, entitled "USE OF COLLECTIONS OF BINDING PROTEINS AND TAGS FOR SAMPLE PROFILING," to U.S. patent application 10/351,011 filed January 24, 2003, to Dana Ault-Riche and Paul D. Kassner, entitled "USE OF COLLECTIONS OF BINDING PROTEINS AND TAGS FOR SAMPLE PROFILING," and to International PCT application No. WO03/062402. This application also is related to U.S. provisional application Serial No. 60/446,687, filed February 10, 2003, to Dana Ault-Riche, Krishnanand D. Kumble, Rainer Schulz and Kenneth Schulz, entitled "SELF-ASSEMBLING ARRAYS AND USES THEREOF." This application also is related to U.S. application Serial No. attorney dkt nos. ~~25885-1754 and 25885-1754PC 10/699,088 and International application Serial No. PCT/US03/34821~~, each entitled "METHODS FOR PRODUCING POLYPEPTIDE-TAGGED COLLECTIONS AND CAPTURE SYSTEMS CONTAINING THE TAGGED POLYPEPTIDES," to U.S. application Serial No. attorney dkt. no. ~~25885-1759PC International application serial no. PCT/US03/34693~~, entitled "SYSTEMS FOR CAPTURE AND ANALYSIS OF BIOLOGICAL PARTICLES AND METHODS USING THE SYSTEMS", and to U.S. application Serial No. attorney dkt. no. ~~25885-1755 and 1755PC~~

No. 10/699,113 and International application Serial No. PCT/US03/34747, each entitled, "SELF-ASSEMBLING ARRAYS AND USES THEREOF", filed the same day herewith.

Please replace the paragraph beginning at page 8, line 11 to page 9, line 28 with the following amended paragraph:

The methods can further include a secondary agent or a plurality thereof at each locus in the capture system, where the secondary agents are common to a plurality of loci, and bind to and/or interact with the captured biological particles. The amounts of the secondary agents can vary from locus to locus. The secondary agent can, for example, serve to anchor the biological particle, to act as a co-stimulatory molecule, to bind to surface receptors different from the capture agents, to exert a biological effect, or to further select the biological particles that bind to a locus. Secondary agents include, but are not limited to, an organic compound, inorganic compound, metal complex, receptor, enzyme, protein complex, antibody, protein, nucleic acid, peptide nucleic acid, DNA, RNA, polynucleotide, oligonucleotide, oligosaccharide, lipid, lipoprotein, amino acid, peptide, polypeptide, peptidomimetic, carbohydrate, cofactor, drug, prodrug, lectin, sugar, glycoprotein, biomolecule, macromolecule, an antibody or fragment thereof, antibody conjugate, biopolymer, polymer or any combination, portion, salt, or derivative thereof. Some exemplary molecules that can serve as secondary agents include, but are not limited to, adhesion molecules ; angiotenin angiogenic factors ; binding proteins ; cell surface proteins , cell surface receptors; chemokines ; chemokine receptors ; cytokines and their receptors ; ephrin and ephrin receptors; epidermal growth factors ; fibroblast growth factors (FGFs) and receptors (FGFRs); platelet-derived growth factors (PDGFs) and receptors (PDGFRs); transforming growth factors beta (TGFs- β), activins, bone morphogenic proteins (BMPs) and receptors (BMPRs), endometrial bleeding associated factor (EBAF), inhibin A and MIC-1; transforming growth factors alpha (TGFs- α); insulin-like growth factors (IGFs); integrins (alphas and betas); interleukins and interleukin receptors; neutrophic factors ; interferons and their receptors; orphan receptors ; proteases and release factors ; T cell receptors; MHC peptides; MHC peptide complexes; B cell receptors; intracellular adhesion molecules

(ICAMs); Toll-like receptors (TLRs); pattern recognitions receptors (PRR receptors) and PPAR ligands (peroxisome proliferative-activated receptors); ion channels receptors; neurotransmitters and their receptors ; muscarinic receptors; small molecule receptors ; steroid hormones and their receptors ; peptide hormones and their receptors ; tumor necrosis factors (TNFs), TNF receptors; nuclear factors; and G proteins and G protein coupled receptors (GPCRs). Secondary agents can also include drugs, such as the anti-Her-2 monoclonal antibody trastuzumab (herceptin[®]) and the anti-CD20 monoclonal antibodies rituximab (rituxan[®]), toatumomab (BexxarTM) (Bexxar[®]) and Ibritumomab (ZevalinTM), (Zevalin[®]), the anti-CD52 monoclonal antibody Alemtuzumab (CampathTM), (Campath[®]), the anti-TNF α antibodies infliximab (RemicadeTM) (Remicade[®]) and CDP-571 (Humicade[®]), the monoclonal antibody edrecolomab (Panorex[®]), the anti-CD3 antibody muromab-CD3 (Orthoclone[®]), the anti-IL-2R antibody daclizumab (Zenapax[®]), the omalizumab antibody against IgE (Xolair[®]), the monoclonal antibody bevacizumab (AvatinTM), (Avatin[®]), small molecules such as erlotinib-HCl (TareevaTM) (Tarceva[®]) and others that bind to receptors or cell surface proteins, antibodies known to bind to the captured biological particles, receptors, enzymes and combinations thereof.

Please replace the paragraph beginning at page 15, line 15-20 with the following amended paragraph:

FIGURES 7A and 7B depict screening for test compounds or ~~eonitions~~ conditions that modulate interactions and screening for test compounds or ~~eonitions~~ conditions that modulate the effect of interactions, respectively. The figures depict different screening methods using capture systems to capture cells in the presence and absence of test compounds and conditions.

Please replace the paragraph beginning at page 34, line 26 to page 35, line 5 with the following amended paragraph:

As used herein, non-radioactive energy transfer reactions, such as FET (~~fluorescent~~ fluorescence energy transfer) assays, FRET (~~fluorescent~~ fluorescence resonance energy transfer) assays, fluorescence polarization (FP) assays and HTRF (homogeneous time-resolved

fluorescence), are homogeneous luminescence assays based on energy transfer and are carried out between a donor luminescent label and an acceptor label (see, e.g., Cardullo *et al.* (1988) *Proc. Natl. Acad. Sci. U.S.A.* 85:8790-8794; Pearce *et al.* (1986) *Proc. Natl. Acad. Sci. U.S.A.* 83:8092-8096; U.S. Patent No. 4,777,128; U.S. Patent No. 5,162,508; U.S. Patent No. 4,927,923; U.S. Patent No. 5,279,943; and International PCT Application No. WO 92/01225).

Please replace the paragraph beginning at page 35, line 6-14 with the following amended paragraph:

As used herein, Fluorescence Resonance Energy Transfer (FRET) refers to non-radiative energy transfer between chemical and/or proteinfluors. Fluorescent Fluorescence resonance energy transfer (FRET) is an art-recognized process in which one fluorophore (the acceptor) can be promoted to an excited electronic state through quantum mechanical coupling with and receipt of energy from an electronically excited second fluorophore (the donor). This transfer of energy results in a decrease in visible fluorescence emission by the donor and an increase in fluorescent energy emission by the acceptor.

Please replace the paragraph beginning at page 67, line 14 to page 68, line 4 with the following amended paragraph:

Capture agent-polypeptide pairs can be identified by analyzing complementarity-determining regions (CDRs) in the antibody of interest. Translation of available cDNA sequences of the variable light and variable heavy chains of a particular antibody permit the delineation of the CDRs by comparison to the database of protein sequences compiled in "Sequences of Proteins of Immunological Interest", Fifth Edition, Volume 1, Editors: Kabat *et al.* (1991) (see, e.g., table on page xvi). In some cases, CDR peptides can mimic the activity of an antibody molecule (Williams *et al.* *Proc. Natl. Acad. Sci. U.S.A.* 86: 5537 (1989)). CDR peptides may bind their cognate antibody, thus effecting displacement of the antibody from the antigen. To increase the efficiency of the above procedures in identifying candidate releasing peptides, biospecific interaction analysis using surface plasmon resonance detection through the

use of the Pharmacia **BIAcore™-BIAcore®** system can be used. This technology provides the ability to determine binding constants and dissociation constants of antibody-antigen interactions. Analysis of multiple antibodies and the number of biopanning steps (at set antibody concentrations) required to identify a tight-binding consensus peptide sequence will provide a database on which to compare kinetic binding parameters with the ability to identify tight binding polypeptide tags. The use of the **BIAcore™-BIAcore®** system requires purified antibody and a source of soluble antigen. Phage display-selected clones can be used as a source of peptide antigen and directly analyzed for antibody binding.

Please replace the paragraph beginning at page 68, line 7-25 with the following amended paragraph:

In silico methods can be used to determine capture agent - polypeptide tag pairs. Structural information (NMR and X-ray) is known for numerous immunoglobulins and is accessible, for example, at the Protein Databank (online at rcsb.org/pdb/) and ImMunoGeneTics (online at imgt.cnusc.fr:8104/home.html). Using one of a number of available molecular modeling programs such as HyperChem HyperChem® (Hypercube, Inc.), InsightII Insight II® (Molecular Simulations, Inc.), SpartanPro (Schrodinger, Inc.) [[Sybyl]] Sybyl® (Tripos, Inc.) and XtalView XtalView® (Tripos, Inc.) the structural data can be manipulated *in silico* to identify potential molecules that can interact with the variable region of the antibody. The energy of interaction between the antibody and potential epitope can be determined using a molecular docking program such as DOCK, which is commercially available; see, also, e.g., (online at cmpharm.ucsf.edu/kuntz/dock.html), AutoDock (online at scripps.edu/pub/olson-web/doc/autodock/), IDock (online at archive.ncsa.uiuc.edu/Vis/Projects/Docker/) or SPIDeR (online at simbiosys.ca/sprout/eccc/spider.html). Once identified and the binding energy is determined *in silico*, polypeptides that constitute the tags can be synthesized or purchased commercially and tested *in vitro* for their specificity and affinity for the antibody in question.

Please replace the paragraph beginning at page 127, line 1-18 with the following amended paragraph:

Molecules, such as antibodies, are commercially available conjugated to a detectable label or are synthetically producible for use in specific staining depending on the particular molecule or class of molecules of interest. Proteins which can be used as a detectable label include, but are not limited to, GFP, RFP and BFP. A wide variety of luminescent molecules are commercially available, and include, but are not limited to, FITC, fluorescein, rhodamine, Cascade Blue Cascade Blue[®], Marina Blue Marina Blue[®], Alexa Fluor[®] 350, red-fluorescent Alexa Fluor[®] 594, ~~Texas Red~~, ~~Texas Red-X~~ Texas Red[®], ~~Texas Red - X~~ and the red- to infrared-fluorescent Alexa Fluor[®] 633, Alexa Fluor[®] 647, Alexa Fluor[®] 660, Alexa Fluor[®] 680, Alexa Fluor[®] 700 and Alexa Fluor[®] 750 dyes (Molecular Probes). Attachment of the luminescent molecule can be performed by any means known to those skilled in the art, such with the Zenon One Mouse IgG1 labeling kit from Molecular Probes. Conjugated antibodies also can be commercially purchased with the luminescent label already attached from companies such as Molecular Probes (online at probes.com), Invitrogen (www.invitrogen.com), Amersham Biosciences (online at amershambiosciences.com) and Pierce Biotechnologies (online at piercenet.com).

Please replace the paragraph beginning at page 141, line 12-29 with the following amended paragraph:

In immunostaining techniques, a luminescent label is a molecule that can be attached to either a primary or secondary antibody and visualized without the addition of a substrate or a chromagen. Proteins which can be used include, but are not limited to, GFP, RFP and BFP. A wide variety of luminescent molecules are commercially available, and include, but are not limited to, FITC, fluorescein, rhodamine, Cascade Blue Cascade Blue[®], Marina Blue Marina Blue[®], Alexa Fluor[®] 350, red-fluorescent Alexa Fluor[®] 594, ~~Texas Red~~, ~~Texas Red-X~~ Texas Red[®], ~~Texas Red - X~~ and the red- to infrared-fluorescent Alexa Fluor[®] 633, Alexa Fluor[®] 647, Alexa Fluor[®] 660, Alexa Fluor[®] 680, Alexa Fluor[®] 700 and Alexa Fluor[®] 750 dyes (Molecular

Probes). Attachment of the luminescent molecule can be performed by any means known to those skilled in the art, such with the Zenon One Mouse IgG1 labeling kit from Molecular Probes. Conjugated antibodies also can be commercially purchased with the luminescent label already attached from companies such as Molecular Probes (online at probes.com), Invitrogen (online at invitrogen.com), Amersham Biosciences (online at amershambiosciences.com) and Pierce Biotechnologies (online at piercenet.com).

Please replace the paragraph beginning at page 150, line 1-28 with the following amended paragraph:

As noted above, LRET refers to non-radiative energy transfer between chemical and/or biological luminescent molecules, such as, but not limited to fluorophores, bioluminescers and phosphorescers (Heim *et al.* *Curr. Biol.* 6:178-182 (1996); Mitra *et al.* *Gene* 173:13-17 (1996); Selvin *et al.* *Meth. Enzymol.* 246:300-345 (1995); Matyus J. *Photochem. Photobiol. B: Biol.* 12: 323-337 (1992); Wu *et al.* *Anal. Biochem.* 218:1-13 (1994)). The type of LRET observed is dependent on the luminescent molecules present in the sample. LRET among fluorophores gives fluorescent fluorescence resonance energy transfer (FRET), among bioluminescent molecules gives bioluminescent resonance energy transfer (BRET) and among phosphorescent molecules gives LRET. The efficiency of LRET is dependent on the inverse sixth power of the intermolecular separation making it useful over distances comparable with the dimensions of biological macromolecules (Stryer and Haugland *Proc Natl Acad Sci U S A* 58: 719-726 (1967)). Thus, LRET is an important technique for investigating a variety of biological phenomena that produce changes in molecular proximity (dos Remedios *et al.* *J Struct Biol* 115: 175-185 (1995); Selvin *Methods Enzymol* 246: 300-334 (1995); Boyde *et al.* *Scanning* 17: 72-85 (1995); Wu *et al.* *Anal Biochem* 218: 1-13 (1994); Van der Meer *et al.* *Resonance Energy Transfer Theory and Data* pp. 133-168 (1994); dos Remedios *et al.* *J Muscle Res Cell Motil* 8: 97-117 (1987); Kawski *Photochem Photobiol* 38: 487 (1983); Stryer *Annu Rev Biochem* 47: 819-846 (1978); Fairclough *et al.* *Methods Enzymol* 48: 347-379 (1978)). When LRET is used as a contrast mechanism, co-localization of proteins and other molecules can be imaged with spatial resolution beyond the

limits of conventional optical microscopy (Kenworthy *Methods* 24: 289-296 (2001); Gordon *et al. Biophys J* 74: 2702-2713 (1998)).

Please replace the paragraph beginning at page 153, line 18 to page 155, line 6 with the following amended paragraph:

Fluorophores include, but are not limited to, fluorescein, fluorescein isothiocyanate, succinimidyl esters of carboxyfluorescein, succinimidyl esters of fluorescein, 5-isomer of fluorescein dichlorotriazine, caged carboxyfluorescein-alanine-carboxamide, Oregon Green Oregon Green® 488, Oregon Green Oregon Green® 514, Lucifer Yellow, acridine Orange, rhodamine, tetramethylrhodamine, Texas Red Texas Red®, propidium iodide, JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide), tetrabromorhodamine 123, rhodamine 6G, TMRM (tetramethylrhodamine, methyl ester), TMRE(tetramethylrhodamine, ethyl ester), tetramethylrosamine, rhodamine B and 4-dimethylaminotetramethylrosamine, green fluorescent protein, blue-shifted green fluorescent protein, cyan-shifted green fluorescent protein, red-shifted green fluorescent protein, yellow-shifted green fluorescent protein, 4-acetamido-4'-isothiocyanostilbene-2,2'disulfonic acid; acridine and derivatives: acridine, acridine isothiocyanate; 5-(2'-aminoethyl)aminonaphthalene-1-sulfonic acid (EDANS); 4-amino-N-[3-vinylsulfonyl]phenyl]naphthalimide-3,5 disulfonate; N-(4-anilino-1-naphthyl)maleimide; anthranilamide; 4,4-difluoro-5-(2-thienyl)-4-bora-3a,4a diaza-5-indacene-3-propioni-c acid BODIPY; Brilliant Yellow; coumarin and derivatives: coumarin, 7-amino-4-methylcoumarin (AMC, Coumarin 120),7-amino-4-trifluoromethylcoumarin (Coumarin 151); cyanine dyes; cyanosine; 4',6-diaminidino-2-phenylindole (DAPI); 5', 5"-dibromopyrogallol-sulfonaphthalein (Bromopyrogallol Red); 7-diethylamino-3-(4'-isothiocyanatophenyl)-4-methylcoumarin; diethylenetriamine pentaacetate; 4,4'-diisothiocyanatodihydro-stilbene-2,2'-disulfonic acid; 4,4'-diisothiocyanostilbene-2,2'-disulfonic acid; 5-(dimethylamino)naphthalene-1-sulfonyl chloride (DNS, dansylchloride); 4-dimethylaminophenylazophenyl-4'-isothiocyanate (DABITC); eosin and derivatives: eosin, eosin isothiocyanate, erythrosin and derivatives: erythrosin B, erythrosin, isothiocyanate; ethidium; fluorescein and derivatives: 5-carboxyfluorescein (FAM),5-(4,6-

dichlorotriazin-2-yl)aminofluorescein (DTAF), 2',7'dimethoxy-4'5'-dichloro-6-carboxyfluorescein (JOE), fluorescein, fluorescein isothiocyanate, QFITC, (XRITC); fluorescamine; IR144; IR1446; Malachite Green isothiocyanate; 4-methylumbellifliferone/ortho cresolphthalein; nitrotyrosine; pararosaniline; Phenol Red; B-phycoerythrin; *o*-phthaldialdehyde; pyrene and derivatives: pyrene, pyrene butyrate, succinimidyl 1-pyrene; butyrate quantum dots; Reactive Red 4 (Cibacron.TM. Brilliant Red 3B-A) rhodamine and derivatives: 6-carboxy-X-rhodamine (ROX), 6-carboxyrhodamine (R6G), lissamine rhodamine B sulfonyl chloride rhodamine (Rhod), rhodamine B, rhodamine 123, rhodamine X isothiocyanate, sulforhodamine B, sulforhodamine 101, sulfonyl chloride derivative of sulforhodamine 101 (Texas Red Texas Red[®]); N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA); tetramethyl rhodamine; tetramethyl rhodamine isothiocyanate (TRITC); riboflavin; 5-(2'-aminoethyl) aminonaphthalene-1-sulfonic acid (EDANS), 4-(4'-dimethylaminophenylazo)benzoic acid (DABCYL), rosolic acid; terbium chelate derivatives; Cy 3; Cy 5; Cy 5.5; Cy 7; IRD 700; IRD 800; La Jolla Blue; phthalo cyanine; and naphthalo cyanine, coumarins and related dyes, xanthene dyes such as rhodols, resorufins, bimanes, acridines, isoindoles, dansyl dyes, aminophthalic hydrazides such as luminol and isoluminol derivatives, aminophthalimides, aminonaphthalimides, aminobenzofurans, aminoquinolines, dicyanohydroquinones, and fluorescent europium and terbium complexes.

Please replace the paragraph beginning at page 155, line 21-27 with the following amended paragraph:

Quenchers that can be used in the methods provided herein include, but are not limited to, diarylrhodamine derivatives, such as the QSY 7, QSY 9, and QSY 21 dyes available from Molecular Probes; dabcyl and dabcyl succinimidyl ester; dabsyl and dabsyl succinimidyl ester; QSY 35 acetic acid succinimidyl ester; QSY 35 iodoacetamide and aliphatic methylamine; ~~Black Hole Quencher~~ Black Holed Quencher[®] dyes from Biosearch Technologies; naphthalate; and Cy5Q and Cy7Q from Amersham Biosciences.

Please replace the paragraph beginning at page 156, line 27 to page 157, line 13 with the following amended paragraph:

These groups can be attached to the molecular and/or biological particle components of the target as a portion of a fusion protein or via a linker. Formation of a fusion protein involves the placement of two separate genes, one encoding the protein of interest and the second encoding the luminescent protein, in sequential order in an appropriate cloning vector, with the stop codon of the first gene removed so that the polymerase continues through the first gene on to the second without disengaging from the template. Several commercial kits are available for the formation of fusion proteins which contain the protein of interest fused to a luminescent protein, including, but not limited to, Green Fluorescent Protein. For example, the GFP Fusion TOPO™ TOPO cloning vector and the pcDNA-DEST47 Gateway™ Gateway vector are available from Invitrogen (Carlsbad, CA) for the expression of a protein of interest fused to GFP. Further, custom designed and assembled genes, including those for fusion protein production, can be commercially ordered and prepared, such as by Sigma Genosys (The Woodlands, TX). Linkers can include affinity interactions, including, but not limited to, multimeric histidine tags and metal complexes, and biotin-avidin interactions.

Please replace the paragraph beginning at page 172, line 13-24 with the following amended paragraph:

The methods provided herein allow for detection of the modulation of cellular apoptosis resulting from the interaction of a biological particle with a capture system. Staining with stains specific for cell viability such as trypan blue or propidium iodide, can be used to determine cell viability after exposure to tagged molecules displayed by the capture system. Necrotic cells are detected by intense propidium iodide staining of the cytoplasm, due to the complete disruption of the plasma membrane. ApopNexin™-ApopNexin® Kits (Serological Corp.) also are used to discriminate apoptotic from necrotic cells, and to label the progression of a cell through the various stages of apoptosis. As apoptosis progresses into the late-stage, the plasma membrane

becomes permeable to DNA dyes such as propidium iodide, which enter the cell and stain yellow/orange.

Please replace the paragraph beginning at page 173, line 3-10 with the following amended paragraph:

For example, cell permeant caspase substrates such as PhiPhiLux®-PhiPhiLux® (OncoImmunin, Inc.); cell permeant caspase 3 and caspase 7 fluorogenic substrates from Molecular Probes; CaspSCREEN Apoptosis Detection Substrate (Chemicon); and CaspaseTag™ CaspaseTag Fluorescein Caspase Activity Kits (Serologicals Inc.) can all be used to monitor production and activity of the caspases. In addition, immunostains, such as anti-active caspase 3 monoclonal antibodies (BD Pharmingen), also are available for detection of apoptosis via the caspases.

Please replace the paragraph beginning at page 173, line 11-23 with the following amended paragraph:

In normal cells, most of the phosphatidylserine (PS) contained in the plasma membrane is oriented towards the cytoplasmic side of the cell membrane. In early stage apoptosis, the cell undergoes surface membrane blebbing, cytoplasmic shrinkage, nuclear DNA fragmentation, chromatin condensation and PS translocation across the plasma membrane to the exposed outer surface of the cell. It is thought that the PS on the membrane surface identifies the cell as a target for destruction by the immune system. ApopNexin™-ApopNexin® Apoptosis Detection Kits (Serological Corp.) exploit this biochemical event using the annexin V protein labeled with either FITC or biotin. Annexin V is a calcium-dependent phospholipid binding protein with a high affinity for PS. In the presence of calcium, annexin V binds rapidly and specifically to PS and is visualized by flow cytometry or microscopy.

Please replace the paragraph beginning at page 173, line 24 to page 174, line 8 with the following amended paragraph:

Mitochondria have the ability to promote apoptosis through release of cytochrome C, which together with Apaf-1 and ATP forms a complex with pro-caspase 9, leading to activation of caspase 9 and the caspase cascade. Bax, and other Bcl-2 proteins, show structural similarities with pore-forming proteins. It has therefore been suggested that Bax can form a transmembrane pore across the outer mitochondrial membrane, leading to loss of membrane potential and efflux of cytochrome C and AIF (apoptosis inducing factor). Fluorescent probes of mitochondrial membrane potential, which drops in apoptotic cells, are available and include, MitoTracker MitoTracker[®] Red, Rhodamine 123, and JC-1 (Molecular Probes); MitoLight MitoLight[®] (Chemicon); and the MitoTag[™] MitoTag[®] JC-1 Assay Kit (Serologicals Corp.). Anti-cytochrome C monoclonal antibodies with a conjugated enzyme or fluorophore also can be used to detect apoptosis. Additional assays for apoptosis stages such as chromatin condensation and fragmentation, are readily available for microscopic detection of DNA fragmentation.

Please replace the paragraph beginning at page 174, line 19 to page 175, line 11 with the following amended paragraph:

To serve as an effective tracer of cell morphology, a fluorescent probe or other detectable molecule can have the capacity for localized introduction into a biological particle, as well as long-term retention within that structure. If used with live cells and tissues, the tracer can be biologically inert and nontoxic. When these conditions are satisfied, the fluorescence or other detectable properties of the tracer can be used to track the position of the tracer over time. A diverse selection of fluorescent tracers, as well as biotinylated, spin-labeled and other tracers are available commercially from Molecular Probes, and include, but are not limited to, cell-permeant cytoplasmic labels (CellTracker Blue CMAC, CellTracker Green CMFDA or CellTracker Orange CMTMR); microinjectable cytoplasmic labels (lucifer yellow CH, ~~Cascade Blue~~ Cascade Blue[®] hydrazide, the Alexa Fluor[®] hydrazides, sulforhodamine 101 and biocytin); membrane tracers (DiI, DiO, DiD, DiR, DiA, R18, FM 1-43, FM 4-64 and their analogs); fluorescent and biotinylated dextran conjugates, fluorescent microspheres (FluoSpheres FluoSpheres[®] and TransFluoSpheres TransFluoSpheres[®] fluorescent microspheres); and

proteins and protein conjugates (Albumin Conjugates, Casein Conjugates, Peroxidase Conjugates, Phycobiliproteins, Fluorescent Histones, and Alexa Fluor® 488 Soybean Trypsin Inhibitor). These tracers can be introduced into the biological particle using any method known to those skilled in the art including, but not limited to, microinjection, hypo-osmotic shock, scrape loading, sonication, high-velocity microprojectiles, glass beads, and electroporation (McNeil, *PL Methods Cell Biol* 29: 153-173 (1989)).

Please replace the paragraph beginning at page 175, line 28 to page 176, line 8 with the following amended paragraph:

Many of fluorescent ligands available first bind to cell surface receptors, then are internalized and, in some cases, recycled to the cell's surface. Consequently, it can be difficult to assess whether the fluorescent signal is emanating from the cell surface, the cell interior or, as is more typical, a combination of the two sites. Furthermore, the fluorophore's sensitivity to environmental factors, principally intracellular pH, can affect the signal of the fluorescent ligand. Molecular Probes has commercially available products by which these signals can be separated and, in some cases, quantitated. For example, antibodies directed to the Alexa Fluor® 488, BODIPY FL, fluorescein/~~Oregon Green~~ Oregon Green®, tetramethylrhodamine, ~~Texas Red~~ Texas Red® and ~~Cascade Blue~~ Cascade Blue® dyes to quench most of the fluorescence of surface-bound or exocytosed probes.

Please replace the paragraph beginning at page 197, line 3-10 with the following amended paragraph:

Non-natural amino acids that are modifications of natural amino acids in the side chain functionality, such that the methylene groups of the side chain of the natural amino acids have been substituted by imino groups or divalent non-carbon atoms or, alternatively, methyl groups have been substituted by amino groups, hydroxyl groups or thiol groups, so as to add ability to form hydrogen bonds or to reduce their hydrophobic properties (e.g. leucine to 2-aminoethylcysteine, or ~~isoleucine~~ isoleucine to o-methylthreonine).

Please replace the paragraph beginning at page 200, line 3-21 with the following amended paragraph:

Non-naturally occurring amino acids can be ranked for antigenicity using methods applied to the naturally occurring amino acids, for example by testing sequences against antisera or libraries of antibodies (described herein) and can be ranked along-side naturally occurring amino acids. For example, a ~~representative~~ representative set of polypeptides composed of non-naturally occurring amino acids and/or a combination of non-naturally occurring and naturally occurring amino acids of a chosen polypeptide length can be used to immunize animals. Based on the subset of polypeptides injected which are antigenic and non-antigenic, amino acids are identified which either are more likely to be present in antigenic polypeptides or are more likely to be present on non-antigenic polypeptides. The likelihood of a amino acid's presence in antigenic polypeptide gives an observed antigenic ranking. Some ~~non-natural~~ non-natural amino acids are very structurally similar to naturally occurring amino acids and to other non-naturally occurring amino acids. This similarity can be factored in to provide antigenicity rankings based on these similarities. Non-naturally occurring amino acids can also be assigned a similarity ranking for use with the methods as described, based on their structural and functional similarity to each other and to naturally occurring amino acids.

Please replace the paragraph beginning at page 200, line 23-31 with the following amended paragraph:

Once the polypeptides are designed, any of the subsets of polypeptides ~~described~~ described herein can be generated by standard methods known in the art. The ~~petides~~ peptides can be chemically synthesized by standard and/or combinatorial chemistry. ~~Polypeptides~~ Polypeptides can also be synthesized using recombinant means such as by expression of nucleic acids encoding the polypeptide sequences. For recombinant expression, the polypeptides are limited to the 20 naturally occurring amino acids and additionally non-naturally occurring amino

acids where the expression organism of choice has been genetically engineered to generate such modifications.

Please replace the paragraph beginning at page 210, line 11-15 with the following amended paragraph:

Eight positive phage clones were picked and added to a 96-deep well plate that contained 100 μ l of E. coli 2738 cells. The plate was incubated at 37°C for 30 min followed by addition of 900 μ l of 2X YT media and an additional incubation at 37°C for 4 hour. This plate then was sent to MJ Research (Waltham, [[CA]] MA) for sequencing.

Please replace the paragraph beginning at page 211, line 30 to page 212, line 22 with the following amended paragraph:

To demonstrate the functioning of the methods herein, capture antibodies, specific, for example, for various peptide epitopes, such as the human influenza virus hemagglutinin (HA) protein epitope, which has the amino acid sequence YPYDVPDYA, were used to tag, for example, scFvs. For example, an scFv with antigen specificity for human fibronectin (HFN) was tagged with an HA epitope, thus generating a molecule (HA-HFN), which was recognized by an antibody specific for the HA peptide and which has antigen specificity of HFN. After depositing various concentrations of the capture antibodies (from 800 μ g/ml to 200 μ g/ml), including anti-HA tag capture antibodies, onto a glass slide coated with a surface for capturing proteins, such as a nitrocellulose-coated slide (FASTTM FAST, Schleicher and Schuell), they were allowed to bind at ambient temperature and humidity of 50 to 60%. After binding, slides with deposited anti-HA capture antibodies were blocked with a protein-containing solution such as Blocker BSATM Blocker BSA (Pierce) diluted to 1X in phosphate-buffered saline (PBS) with [[Tween]] TWEEN-20 (polyoxyethylenesorbitan monolaurate; Sigma) added to a final concentration of 0.05% (vol:vol) or with a 3% non-fat milk in the same buffer to eliminate background signal generated by non-specific protein binding to the membrane. For subsequent description contained herein PBS with 0.05% (vol:vol) [[Tween]] TWEEN-20 is referred to as PBS-T.

Blocking times can be varied from 60 min at ambient temperature to longer hours at ambient temperature or at 4°C, for example. Incubation temperatures for all subsequent steps can be varied from ambient temperature to about 37°C. In all instances, the precise conditions are determined empirically.

Please replace the paragraph beginning at page 213, line 4-16 with the following amended paragraph:

Membranes with deposited anti-HA capture antibodies and bound HA-HFN scFv then were incubated with, for purposes of demonstration, biotinylated human fibronectin (Bio-HFN), which is an antigen that will be recognized by the capture HA-HFN scFv. Bio-HFN was serially diluted (*e.g.*, from 1 to 10 μ g/ml) in BBSA-T. The resulting membranes were washed as before and then were incubated with Neutravidin•HRPO (Pierce) diluted 1 in 10000 in BBSA-T. The resulting slides were washed as before, rinsed with PBS and developed with a 1:1 mixture of freshly prepared Supersignal™ SuperSignal® ELISA Femto Stable Peroxide Solution and Supersignal™ SuperSignal® ELISA Femto Lumino Enhancer Solution (Pierce), and then imaged using an imaging system, such as, for example, a Kodak Image Station 440CF or IS1000 or other such imaging system. A small volume of the Supersignal SuperSignal® solution was plated on the platen of the image station.

Please replace the paragraph beginning at page 219, line 6-10 with the following amended paragraph:

The reactions were mixed gently then spun briefly. The tubes then were set in the thermal cycler preheated to 94°C and the following cycle was started: 94°C for 2 min, 94°C for 1 min, 55°C for 1 min, 72°C for 1 min, 72°C for 10 min for 30 cycles and then held at 4°C. The reactions then were spun briefly and proceed to gel purification [[steps]] steps.

Please replace the paragraph beginning at page 228, line 18 to page 229, line 8 with the following amended paragraph:

Cultures were screened for reactivity to antigen in a standard ELISA. Briefly, 96-well polystyrene plates were coated overnight with 10 μ g/ml antigen (Sigma) in 0.1 M NaHCO₃, pH 8.6 at 4°C. Plates were rinsed twice with 50 mM Tris, 150 mM NaCl, 0.05% [[Tween]] TWEEN-20, pH 7.4 (TBST), and then blocked with 3% non-fat dry milk in TBST (3% NFM-TBST) for 1 hour at 37°C. Plates were rinsed 4 times with TBST and 40 μ l of unclarified culture was added to wells containing 10 μ l 10% NFM in 5X PBS. Following incubation at 37°C for 1 hour, plates were washed 4 times with TBST. The 9E10 monoclonal antibody (Covance) recognizing the myc polypeptide tag was diluted to 0.5 μ g/ml in 3% NFM-TBST and incubated in wells for 1 hour at 37°C. Plates were washed 4 times with TBST and incubated with horseradish peroxidase conjugated goat-anti-mouse IgG (Jackson Immunoresearch, 1:2500 in 3% NFM-TBST) for 1 hour at 37°C. After 4 additional washes with TBST, the wells were developed with *o*-phenylene diamine substrate (Sigma, 0.4mg/ml in 0.05 Citrate phosphate buffer pH 5.0) and stopped with 3N HCl. Plates were read in a microplate reader at 492nm. Cultures eliciting a reading above 0.5 OD units were scored positive and retested for lack of reactivity to a panel of additional antigens. Those clones that lacked reactivity to other antigens, and repeat reactivity to the specific antigen were grown up in culture. The DNA was prepared and the scFv was subcloned by standard methods into the pBADHA and pBADM2 vectors.

Please replace the paragraph beginning at page 233, line 18-23 with the following amended paragraph:

Ten μ L of 10% [[Tween]] TWEEN -20 solution was added to each elution tube. The eluate then was added to a dialysis cassette, which was placed in 1 L of phosphate buffered saline, pH 7.4 (PBS). The buffer exchange was allowed to take place overnight with stirring at 4°C. Glycerol was added to each dialyzed sample to a final concentration of 20% and each sample was aliquoted and stored at -80°C.

Please replace the paragraph beginning at page 233, line 27 to page 234, line 4 with the following amended paragraph:

The components of Enzyme-linked immunosorbent assay (ELISA) CytoSets™-CytoSets kits (BioSource), available for the detection of human cytokines, were used to generate “sandwich assays” for certain experiments. The “sandwich” as used in the below description was composed of a bound capture antibody, a purified cytokine antigen, a detector antibody, and streptavidin•HRPO. These kits allowed for the detection of the following human cytokines: human tumor necrosis factor alpha (Hu TNF- α ; catalog # CHC1754, lot # 001901) and human interleukin 6 (Hu IL-6; catalog # CHC1264, lot # 002901).

Please replace the paragraph beginning at page 234, line 14-22 with the following amended paragraph:

1. **Preparation of CytoSets™-CytoSets capture antibodies for printing with either a modified inkjet printer or a pin-style microarray printer**

Prior to printing CytoSets™-CytoSets antibodies using a modified inkjet printer or a pin-style microarray printer (see below), capture antibodies from these kits were diluted in glycerol (Sigma catalog # G-6297, lot # 20K0214) to 1-2 mg/ml, in a final glycerol concentration of 1% or 10%. Typically these mixtures were made in bulk and stored in microcentrifuge tubes at 4°C.

Please replace the paragraph beginning at page 235, line 5-12 with the following amended paragraph:

CytoSets™-CytoSets capture antibodies were printed with an inkjet printer (Canon model BJC 8200 color inkjet) modified for this application. The six color ink cartridges were first removed from the print head. One-milliliter pipette tips then were cut to fit, in a sealed fashion, over the inkpad reservoir wells in the print head. Various concentrations of capture antibodies, in glycerol, then were pipetted into the pipette tips which were seated on the inkpad reservoirs (typically the pad for the black ink reservoir was used).

Please replace the paragraph beginning at page 235, line 13-24 with the following amended paragraph:

For generation of printed images using the modified printer, Microsoft PowerPoint was used to create various on-screen images in black-and-white. The images then were printed onto nitrocellulose paper (Schleicher and Schuell (S&S) Protran BA85, pore size 0.45 μ m, VWR catalog # 10402588, lot # CF0628-1) which was cut to fit and taped over the center of an 8.5 x 11 inch piece of printer paper. This two-paper set was hand fed into the printer immediately prior to printing. After printing of the image, the antibodies were dried at ambient temperature for 30 min. The nitrocellulose then was removed from the printer paper, and processed as described below (see Basic protocol for antibody and antigen incubations: ~~FAST~~TM FAST slides and nitrocellulose filters printed with CytoSetsTM-CytoSets capture antibodies).

Please replace the paragraph beginning at page 235, line 27 to page 236, line 17 with the following amended paragraph:

Capture antibody dilutions were printed onto nitrocellulose slides (Schleicher and Schuell ~~FAST~~TM FAST slides; VWR catalog # 10484182, lot # EMDZ018) using a pin-printer-style microarrayer (MicroSys 5100; Cartesian Technologies; TeleChem ArrayItTM ArrayIt[®] Chipmaker 2 microspotting pins, catalog # CMP2). Printing was performed using the manufacturer's printing software program (Cartesian Technologies' AxSys version 1, 7, 0, 79) and a single pin (for some experiments), or four pins (for some experiments). Typical print program parameters were as follows: source well dwell time 3 sec; touch-off 16 times; microspots printed at 0.5 mm pitch; pins down speed to slide (start at 10 mm/sec, top at 20 mm/sec, acceleration at 1000 mm/sec²); slide dwell time 5 millisec; wash cycle (2 moves + 5 mm in rinse tank; vacuum dry 5 sec); vacuum dry 5 sec at end. Microarray patterns were pre-programmed (in-house) to suit a particular microarray configuration. In many cases, replicate arrays were printed onto a single slide, allowing subsequent analyses of multiple analyte parameters (as one example) to be performed on a single printed slide. This in turn maximized the amount of experimental data generated from such slides. Microtiter plates (96-well for most

experiments, 384-well for some experiments) containing capture antibody dilutions were loaded into the microarray printer for printing onto the slides. Based on the reported print volume (post-touch-off, see above) of 1 nl/microspot for the Chipmaker 2 pins, the capture antibody concentrations contained in the printed microspots typically ranged from 800 to 6 pg/microspot.

Please replace the paragraph beginning at page 236, line 26 to page 237, line 2 with the following amended paragraph:

Following capture antibody printing, blocking of slides was performed with ~~Bloeker BSA™-Blocker BSA~~ (10% or 10X stock; Pierce catalog # 37525) diluted in phosphate-buffered saline (PBS) (~~BupH™ BupH~~ modified Dulbecco's PBS packs; Pierce catalog # 28374). ~~[[Tween]]TWEEN~~-20 (polyoxyethylene-sorbitan monolaurate; Sigma catalog # P-7949) then was added to a final concentration of 0.05% (vol:vol). The resulting blocker is hereafter referred to as BBSA-T, while the resulting PBS with 0.05% (vol:vol) ~~[[Tween]]TWEEN~~-20 is referred to as PBS-T.

Please replace the paragraph beginning at page 237, line 3-12 with the following amended paragraph:

E. Incubation Chamber Assemblies for ~~FAST™ FAST~~ Slides

For isolation of individual microarrays of capture antibodies on a single ~~FAST™ FAST~~ slide, slotted aluminum blocks were machined to match the dimensions of the ~~FAST™ FAST~~ slides. Silicone isolator gaskets (Grace BioLabs; VWR catalog #s 10485011 and 10485012) were hand-cut to fit the dimensions of the slotted aluminum blocks. A "sandwich" consisting of a printed slide, gasket, and aluminum block then was assembled and held together with 0.75 inch binder clips. The minimum and maximum volumes for one such isolation chamber, isolating one antibody microarray, were 50 and 200 μ l, respectively.

Please replace the paragraph beginning at page 237, line 14-21 with the following amended paragraph:

1. FAST™ FAST Slides and Nitrocellulose Filters Printed with CytoSets™ CytoSets Capture Antibodies

After printing CytoSets™ CytoSets capture antibodies onto FAST™ FAST slides or nitrocellulose filters, these support media were allowed to dry as described. Slides and filters then were blocked with BBSA-T, for 30 min to 1 hr, at ambient temperature (filters) or 37°C (slides). All incubations were done on an orbital table (ambient temperature incubations) or in a shaking incubator (37°C incubations).

Please replace the paragraph beginning at page 237, line 22-32 with the following amended paragraph:

Purified, recombinant cytokine antigen (contained in each CytoSets™ CytoSets kit) then was diluted to various concentrations (typically between 1-10 ng/ml) in BBSA-T. Slides or filters, containing CytoSets™ CytoSets capture antibodies, then were incubated with this antigen solution at ambient temperature (filters) or 37°C (slides). Slides and filters then were washed three times with PBS-T, 3-5 min per wash, at ambient temperature. These slides and filters, containing capture antibody with bound antigen, then were incubated with detector antibody (contained in each kit) diluted 1:2500 in BBSA-T for 1hr, at ambient temperature (filters) or 37°C (slides). Slides and filters then were washed with PBS-T as described above.

Please replace the paragraph beginning at page 238, line 7-12 with the following amended paragraph:

2. FAST™ FAST Slides Printed with Anti-peptide Tag Capture Antibodies

After printing anti-peptide tag capture antibodies onto FAST™ FAST slides, the slides were allowed to dry as described. Slides then were blocked with BBSA-T, for 30 min to 1 hr, at 37°C in a shaking incubator (37°C incubations).

Please replace the paragraph beginning at page 239, line 1-9 with the following amended paragraph:

G. Developing and Imaging of FASTTM FAST Slides and Nitrocellulose Filters Containing Antibody Microarrays

After washing in PBS-T, slides containing anti-peptide tag antibodies, bound scFvs, antigens, and Neutravidin•HRPO, or nitrocellulose filters containing CytoSetsTM CytoSets antibodies, bound cytokine antigens, detector antibody, and streptavidinHRPO, were rinsed with PBS, then developed with SuperSignalTM-SuperSignal[®] ELISA Femto Stable Peroxide Solution and SuperSignalTM-SuperSignal[®] ELISA Femto Luminol Enhancer Solution (Pierce catalog # 37075) following the manufacturer's recommendations.

Please replace the paragraph beginning at page 239, line 10-25 with the following amended paragraph:

FASTTM FAST slides and filters were imaged using the Kodak Image Station 440CF. A 1:1 mixture of peroxide solution:luminol was prepared, and a small volume of this mixture was placed onto the platen of the image station. Slides then were placed individually (microarray-side down) into the center of the platen, thus placing the surface area of the nitrocellulose-containing portion of the slide (containing the microarrays) into the center of the imaging field of the camera lens. In this way the small volume of developer, present on the platen, contacted the entire surface area of the nitrocellulose-containing portion of the slide. Nitrocellulose filters were treated in the same manner, using somewhat larger developer volumes on the platen. The Image Station cover then was closed and microarray images were captured. Camera focus (zoom) was set to 75mm (maximum; for FASTTM FAST slides) or 25mm for filters. Exposure times ranged from 30 sec to 5 min. Camera f-stop settings ranged from 1.2 to 8 (Image Station f-stop settings are infinitely adjustable between 1.2 and 16).

Please replace the paragraph beginning at page 240, line 8-18 with the following amended paragraph:

Two microarray-type patterns of human tumor necrosis factor α (TNF- α) capture antibody (from CytoSetsTM CytoSets kit) were printed onto nitrocellulose with a modified inkjet printer using Microsoft PowerPoint. TNF- α capture antibody was diluted to 1.25 ng/ml in 1% glycerol for printing. After drying, the filter was blocked with BBSA-T. The microarrays then were probed with purified recombinant human TNF- α (5.65 ng/ml) as antigen. The filter then was washed with PBS-T. Detector antibody and streptavidin-HRPO then were used for detection of bound antigen. After washing in PBS-T, the microarrays were developed using chemiluminescence and imaged on a Kodak Image Station 440CF. High resolution images were generated with feature sizes below 50 μ m.

Please replace the paragraph beginning at page 240, line 19 to page 241, line 5 with the following amended paragraph:

A single microarray of human interleukin-6 (IL-6) capture antibody (from CytoSetsTM CytoSets kit) was printed onto a FASTTM FAST slide with a pin-style microarray printer (4-pin print pattern) programmed to print the pattern. IL-6 capture antibody was diluted to 0.5 mg/ml in 10% glycerol. One nanoliter microspots of capture antibody were printed which contained 500 pg/microspot. After drying, the slide was blocked with BBSA-T. The microarray then was probed with purified recombinant human IL-6 (5 ng/ml) as antigen. Following incubation with the antigen, the slide was washed with PBS-T. Detector antibody and streptavidin-HRPO then were used for detection of bound antigen. After washing in PBS-T, the microarrays were developed using chemiluminescence and imaged on a Kodak Image Station 440CF. The method produced bright images with array feature sizes corresponding to 300 μ m loci. In additional experiments, dilution of capture antibody or antigen gave increased or reduced signals corresponding to a direct relationship between the amount of antigen bound and the signal produced.

Please replace the paragraph beginning at page 241, line 7-25 with the following amended paragraph:

Microarrays (8-by-8 microspots) of anti-peptide tag capture antibodies (HA.11, specific for the influenza virus hemagglutinin epitope YPYDVPDYA; 9E10, specific for the EQKLISEEDL (SEQ ID No. 91) amino acid region of the Myc oncoprotein; and FLOPC-21, a negative control antibody of unknown specificity) were printed onto a FASTTM FAST slide with a pin-style microarray printer (4-pin print pattern) programmed to print the pattern. The capture antibodies were diluted to 0.5 mg/ml in 20% glycerol. One nanoliter microspots were printed which contained serial two-fold dilutions of 500, 250, 125 and 62.5 pg/microspot. After drying, the filter was blocked with BBSA-T. The microarrays then were successively probed with aliquots of culture supernatant and periplasmic lysate harvested from an *E. coli* strain harboring the plasmid construct which directs the expression of the HA-HFN scFv upon arabinose induction. The slide then was washed with PBS-T. The microarrays then were probed with biotinylated human fibronectin (3.3 μ g/ml). After washing with PBS-T, the microarrays were probed with excess Neutravidin \bullet HRPO (1:1000). After washing in PBS-T, the microarrays were developed using chemiluminescence and imaged on a Kodak Image Station 440CF.

Please replace the paragraph beginning at page 241, line 27 to page 242, line 11 with the following amended paragraph:

Microarrays of human interleukin-6 (IL-6) capture antibody (from CytoSetsTM CytoSets kit) were printed onto a FASTTM FAST slide, and 4 different surfaces, with a pin-style microarray printer (4-pin print pattern) programmed to print the pattern. Human IL-6 capture antibody was diluted in 20% glycerol and printed to yield serial three-fold dilutions ranging from 300, 100, 33, 11, 3.6, 1, 0.3, and 0.1 pg/microspot. A negative control capture antibody, specific for human interferon- α (IFN- α) was also printed at 50 pg/microspot. After drying, the slide was blocked with BBSA-T. The microarrays then were probed with purified recombinant human IL-6 (5 ng/ml) as antigen followed by washing with PBS-T. Detector antibody and streptavidin \bullet HRPO then were used for detection of bound antigen. After washing in PBS-T, the microarrays were developed using chemiluminescence and imaged on a Kodak Image Station 440CF. Signal was seen from loci containing 1 pg/locus and higher concentrations.

Please replace the paragraph beginning at page 243, line 9-17 with the following amended paragraph:

An equal volume of 2X Print Buffer (2X PBS, 40% glycerol and 0.002% [[Tween]]TWEEN-20) was added to each of the scFv sub-libraries to a final volume of 40 μ l in a 96-well PCR plate. The solution was mixed and then spun briefly. The array libraries were printed on nitrocellulose-coated glass slides (FASTTM FAST, Schleicher and Schuell, NH) using Telechem pins (CM-2) on a Cartesian printer (MicroSys 5100) such that 20 replicate arrays were printed on each slide. Printing was performed under 55 to 60% humidity and the plates air-dried for 1 hour followed by storage at 4°C.

Please replace the paragraph beginning at page 244, line 2-21 with the following amended paragraph:

Capture anti-tag antibodies were printed at 800, 200, and 50 μ g/ml in ten replicate arrays onto n/10 FASTTM FAST slides (where n= number of scFv pools to be analyzed). An extra slide was printed for use in obtaining the standard curve. Slides were incubated in Blocking solution (5% non-fat milk in PBS containing 0.1% [[Tween]]TWEEN-20) for 1 hour at 37°C. Each pool of scFv was diluted to appropriate concentration (typically between 1 and 10 μ g/ml) in Blocking Buffer and incubated with individual arrays for 1 hour at room temperature. A standard curve was generated with known amounts of scFV:huFN:tag (scFv recognizing human fibronectin conjugated to individual tags) by serial dilutions onto one slide so that samples can be quantified. Unbound scFv were removed by aspiration and slides were washed three times with Blocking solution. Rabbit anti - Hiss6 polyclonal antibody conjugated to HRP was incubated with all arrays at a 1:20,000 dilution from a 1 mg/ml stock solution for 30 minutes at room temperature. Slides were washed with PBS containing 0.1% [[Tween]]TWEEN-20, prior to the addition of Luminol for detection on a Kodak IS1000 imaging station. The intensity of each locus was measured and the amount of individual tagged scFv in each pool determined by measuring against the standard curve.

Please replace the paragraph beginning at page 245, line 4-8 with the following amended paragraph:

Each of the antibodies were printed on ~~FAST~~TM FAST nitrocellulose - coated glass slides (Schleicher and Schuell) using a Telechem pin (CM-2) in a Cartesian printer (MicroSys 5100). Printing was performed at 55 to 60% relative humidity. The slides were subsequently incubated overnight at 4°C for maximum adsorption to the nitrocellulose.

Please replace the paragraph beginning at page 248, line 24-28 with the following amended paragraph:

Each of the antibodies were printed on ~~FAST~~TM FAST nitrocellulose - coated glass slides (Schleicher and Schuell) using a Telechem pin (CM4) in a Cartesian printer (MicroSys 5100). Printing was performed at 55 to 60% relative humidity. The slides were subsequently incubated overnight at 4°C for maximum adsorption to the nitrocellulose.

Please replace the paragraph beginning at page 256, line 8-13 with the following amended paragraph:

Each of the antibodies were printed in ten arrays on four ~~FAST~~TM FAST nitrocellulose - coated glass slides (Schleicher and Schuell) using a Telechem pin (CM4) in a Cartesian printer (MicroSys 5100). Printing was performed at 55 to 60% relative humidity. The slides were subsequently incubated overnight at 4°C for maximum adsorption to the nitrocellulose and then stored at 4°C until use.

Please replace the paragraph beginning at page 259, line 20-24 with the following amended paragraph:

The Fluorescence Labeling Solution was prepared as follows: Goat anti-Mouse IgM - Oregon Green Oregon Green[®] (Molecular Probes) was diluted in Block Buffer to a final

concentration of 5 μ g/ml. Five μ l per 200 μ l of Fluorescence Labeling Solution of Rhodamine - Phalloidin (Molecular Probes) then was added from a stock (300 Units/ml).

Please replace the paragraph beginning at page 260, line 7-15 with the following amended paragraph:

Arrays were printed with anti-tag antibodies (800, 200, and 50 μ g/ml solution were printed) and loaded with anti-cell surface receptor scFv fused to the appropriate tag (1 μ g/ml solution). The cells were fixed in a 4% formaldehyde solution, permeabilized with TX-100 and double-fluorescently labeled for both an intracellular protein, actin, as well as a cell surface receptor, membrane-bound IgM. Actin was visualized with Rhodamine and the IgM with ~~Oregon Green~~ Oregon Green[®] fluorescent dye. In the bottom panel, the cells were imaged by differential interference contrast microscopy.

Please replace the paragraph beginning at page 260, line 18-23 with the following amended paragraph:

Capture antibody arrays can be printed into 96-well plate format and used in a similar manner to arrays printed onto ~~FAST~~TM FAST slides and nitrocellulose filters. This example demonstrates the use of the 96-well plate format to assay the Tag distribution in an scFv Tag library. Other assays, including functional assays, are performed in 96-well plate arrays in a similar manner/

Please replace the paragraph beginning at page 260, line 25 to page 261, line 2 with the following amended paragraph:

Capture antibody dilutions were printed onto 96-well Maxisorp Immunoplates (NUNC; catalog #442404) using a pin-printer-style microarrayer (MicroSys 5100; Cartesian Technologies; TeleChem ~~ArrayIt~~TM-ArrayIt[®] Chipmaker 2 microspotting pins, catalog # CMP2). Printing was performed using the manufacturer's printing software program (Cartesian Technologies' AxSys version 1, 7, 0, 79) and a single pin. Microarray patterns were pre-

programmed (in-house) to suit a particular microarray configuration, for example as a 5 X 5 pattern of 35 spots per well in each of 96 wells.

Please replace the paragraph beginning at page 262, line 9-15 with the following amended paragraph:

Tag libraries were prepared using the tags corresponding to the antibodies in the source plate above (wells 2-11). The tag libraries were prepared and purified as in Example 3. A master mix of Tag Library standards was prepared based on the least concentrated of the 10 purified tag libraries such that the final concentration of each Tag library in the mix was 10 μ g/ml in BBSA (~~Bloeker BSA~~TM Blocker BSA; Pierce catalog # 37525).

Please replace the paragraph beginning at page 263, line 1-9 with the following amended paragraph:

Supersignal SuperSignal[®] ELISA Femto Reagents (Pierce) were prepared by mixing the two developer components in equal volumes. Fifty microliters of developer was added to each well of the capture agent-tag library plates. Each plate then was imaged on a Kodak Image Station 440 using pre-set image parameters for half-plate imaging as specified by the manufacturer (Kodak, Rochester, NY). Images were saved as JPEG files and archived for processing and then processed using a software analysis imaging program. The experimental data was plotted relative to standard curves to obtain the relative amounts of each tag in the Tag library.

Please replace the paragraph beginning at page 265, line 16-21 with the following amended paragraph:

After the third wash, the vacuum was applied to dry the resin. A new 96-well deep-well block was put into the vacuum chamber. Elution buffer (50 mM NaH₂PO₄ pH 8.0, 1.5 M NaCl and 500 mM imidazol imidazole) then was applied to the filter block, 150 μ l per well and

Applicant : Ault-Riche et al.
Serial No. : 10/699,114
Filed : October 30, 2003
Preliminary Amendment
Page : 30 of 42

Attorney's Docket No.: 17102-010001 / 1759

allowed to sit for 1 minute. Vacuum then was applied and then an additional 150 μ l of elution buffer was applied and eluted in the same manner.